THE REPETITION OF PATTERN IN THE RESPIRATION OF UCA PUGNAX

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Persistent rhythms of O_2-consumption for two species of fiddler crabs, *Uca pugnax* and *Uca pugilator*, were described by Brown, Bennett and Webb in 1954. Analyses of the data revealed rhythms of several different periods including diurnal, semi-lunar and lunar ones. Of particular interest, from the point of view of the mechanism by which biological rhythms are maintained, is the observation that two rhythms of such similar periods as 24.0 hours (diurnal) and 24.8 hours (lunar-day) persist in an organism under constant conditions. The present investigation has been carried out in an attempt to characterize these two rhythms in terms of the regularity of period and of form, and to investigate the persistence of the lunar-day rhythm under conditions in which the ordinary tidal effects were absent from the environment.

Materials and Methods

All of the animals used in these experiments were specimens of *Uca pugnax* collected at Chappoquoit Beach, Cape Cod, Mass. The animals were transported to the Marine Biological Laboratory in open containers and were kept there in enamel pans with a small amount of sea water.

O_2-consumption was measured by means of Brown respirometers (Brown, 1954), modified as described by Brown (1957). Four respirometer vessels were attached to a recording unit and the whole assembly placed in a sealed barostat. The barostat was evacuated to a pressure of 28.5 inches of mercury, which was somewhat below the expected minimum barometric pressure. A maximum of six such units was in operation at any one time. The barostats were opened at approximately three-day intervals at which time the ammonia and CO_2 absorbents were changed, the oxygen supply was replenished, and fresh animals were placed in the vessels. The barostats themselves were contained in water baths kept at a constant temperature of 24° C. and they were located in a room without windows and provided with constant illumination such that the illumination within the barostats was less than one foot-candle. The lever system of the recording units was such that the recording arms were displaced 1.2 mm. for each gram of weight increase of the respirometer vessels. The ink-writing pen of the recorder traced on millimeter graph paper which was marked off in hours after being removed from the drum. The displacement values for each hour were then recorded.

The methods by which the data so obtained were analyzed are described in the following section.

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Results and Analyses

Hourly rates of oxygen consumption, when calculated as mean values for single days, reveal a range for the summer of 1957 of from 28 to 69 ml./kg./hr. The mean rate of oxygen consumption for the first lunar period of 1956 was found to be 32.4 ± 8.4 ml./kg./hr.; for the second lunar period it was 36.0 ± 6.9 ml./kg./

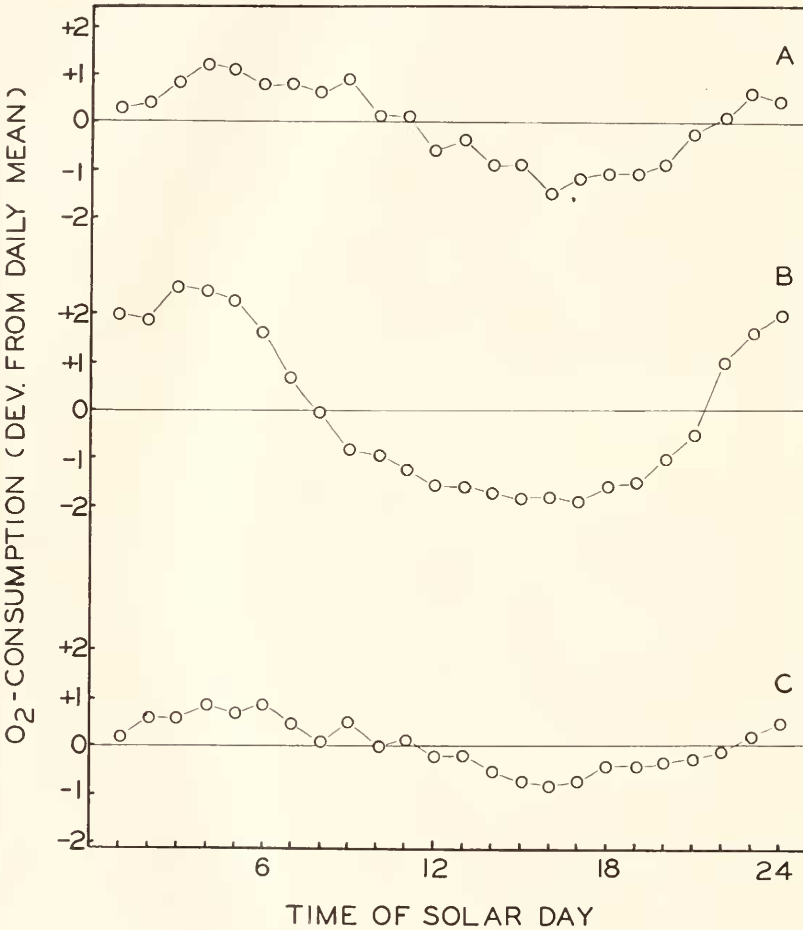

FIGURE 1. The mean diurnal variation in O_2-consumption of *Uca pugnax* for three 29-day periods, (A) July 15 to Aug. 13, 1955, (B) July 13 to Aug. 11, 1956, and (C) July 14 to Aug. 12, 1957.

hr. In the summer of 1957 the comparable values were 41.9 ± 9.4 ml./kg./hr. and 44.6 ± 11.9 ml./kg./hr.

In Figure 1 are seen the average diurnal curves for representative periods in three successive years. Each curve represents the mean hourly values (expressed as deviations from the mean) for a period of 29 days. Figure 1A represents data from two recording units for the period July 15 to August 13, 1955. Figure 1B shows similar values from five recorders for the period July 13 to August 11, 1956. Figure 1C shows the mean daily curve for the period July 14 to August 12, 1957. In this year six recorders were used with a minimum of two on any one day.

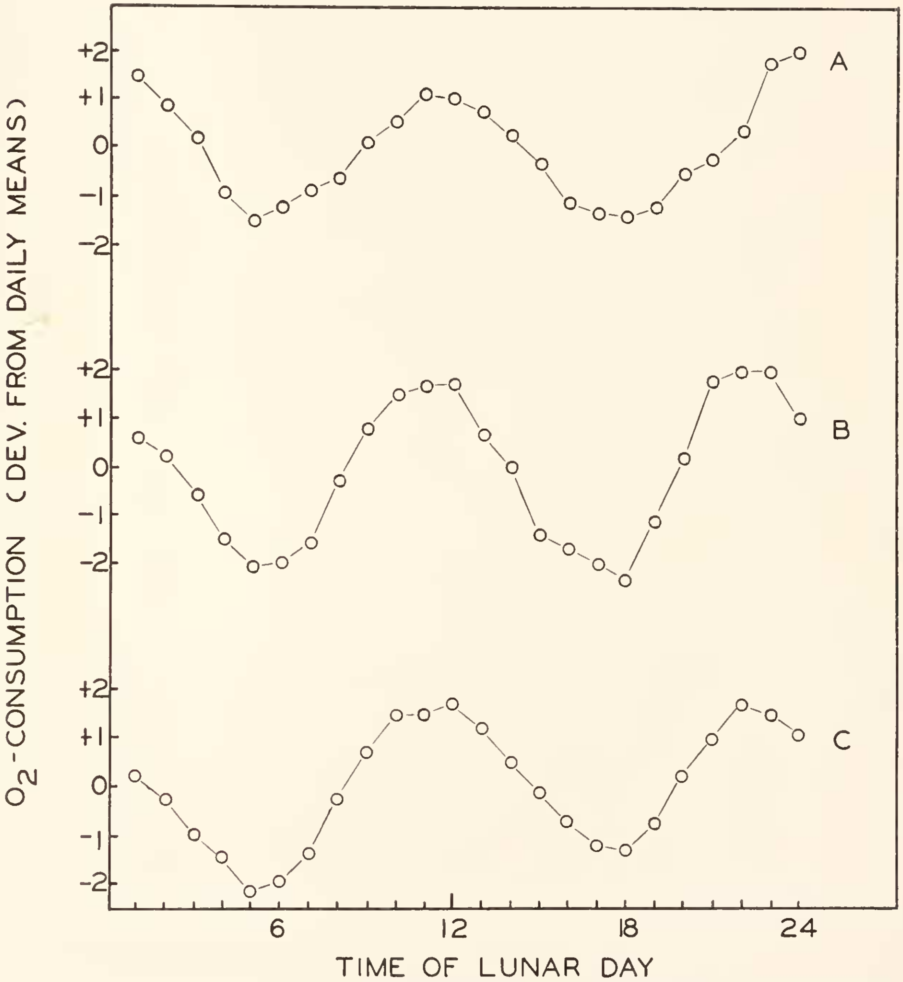

FIGURE 2. The primary lunar rhythm in O_2-consumption of *Uca pugnax* for three 29-day periods. (A) July 15 to Aug. 13, 1955, (B) July 13 to Aug. 11, 1956, and (C) July 14 to Aug. 12, 1957.

In all three cases the diurnal curve is characterized by a single maximum between 2 AM and 6 AM, then a broad minimum extending from about noon to 7 PM, after which there is an increase in rate that continues until midnight. In 1956 (Fig. 1B) the amplitude is greater than in the other two years, but the form and phase relations appear to be essentially the same in all three. The amplitude of the mean diurnal rhythm can be described by the ratio of maximum to minimum value. Expressed in these terms, the mean amplitude obtained in 1956 was 1.4, while that for 1957 was 1.2.

In Figure 2 are shown the primary lunar curves for the periods represented in Figure 1. The curves in Figure 2 were obtained by rendering random the diurnal variations while events of a lunar frequency were kept constant (see Brown, Bennett and Webb, 1954). The points are plotted in such a way that lunar zenith is at 12 hours and lunar nadir is at 24 hours. Figure 2A represents the data from 1955, Figure 2B those from 1956, and Figure 2C those from 1957. The similarity among the primary lunar curves for these three years is even more striking than that exhibited by the diurnal curves, since the likeness now includes amplitude as well as form and phase relationships. The ratio of maximum to minimum for the lunar rhythm remains at about 1.4 in all three years.

All of the curves in Figure 2 show two maxima and two minima. The peak rates of oxygen consumption are seen to occur at approximately lunar zenith and lunar nadir. Both maxima are about the same height and there is similarly little difference between the two minima in a lunar day. Since there appears to be little difference between events occurring at the time of lunar zenith and those at lunar nadir the effect is of a rhythm with a period of about 12 hours. Further, since the amplitude of the lunar rhythm is at least as great as that of the diurnal one (and for 1957 it is considerably greater), one would expect that the curves for respiration on single days would exhibit the lunar component rather prominently and that the form of the daily curves would tend to repeat at approximately 15-day intervals.

A direct and elementary test for 15-day repetition of form is possible from the data presented in Figure 3. In this figure each point represents the average of all machines recording on the particular day. The ordinate values are the displacement in mm. of the recording levers. The number of measurements contributing to each point ranges from three to six. All of the data are from the summer of 1957 and the days represented are as follows: Curve A, for June 24, is the third day before new moon; Curve B, for July 2, the fifth day after new moon; Curve C, July 8, the third day before full moon; Curve D, July 16, the fifth day after full moon; Curve E, July 23, the third day before new moon; Curve F, July 31, the fifth day after new moon; Curve G, August 7, the third day before full moon; Curve H, August 15, the fifth day after full moon; Curve I, August 22, the third day before new moon; and Curve J, August 30, the fifth day after new moon. Thus, reading across the figure, Curves A, E, and I are synchronous with respect to lunar period; each represents the third day before new moon. Curves B, F, and J are synchronous, each representing the fifth day after new moon. Curves C and G both represent the third day before full moon, while Curves D and H represent the fifth day after full moon. If one takes a semi-lunar rather than a lunar period, then alternate curves throughout the figure are synchronous. Thus, Curves A, C, E, G, and I are effectively synchronous in semi-lunar periods.

Examination of Figure 3 shows that on each of the days represented there are fluctuations such that maximal values represent two to three times the minimal values for the day. Two maxima and two minima occur daily, dividing the day roughly into quarters. It is also obvious that the curves can readily be divided into two classes: those which exhibit a maximum in the hours between 6 AM and 12 N and a minimum between 12 N and 6 PM, and those which show a minimum between 6 AM and 12 N and a maximum between 12 N and 6 PM. Curves A, C, E, G, and I fall into the first category and all of the others into the second. In the period immediately preceding new moon (*e.g.*, curve A) lunar zenith occurs in the late morning hours, while as full moon approaches (*e.g.*, curve C) lunar zenith will be in the late evening hours. The data presented in Figure 3 give no evidence that there is any consistent difference between a semi-lunar period including new moon and one including full moon. The relative heights of respiratory maxima on any day seem not to be greatly affected by the time of day at which lunar zenith occurs. These data support the description derived from the mean lunar day curves. The times of lunar zenith and lunar nadir are clearly indicated in the respiratory data for single days, and these times are indicated by major

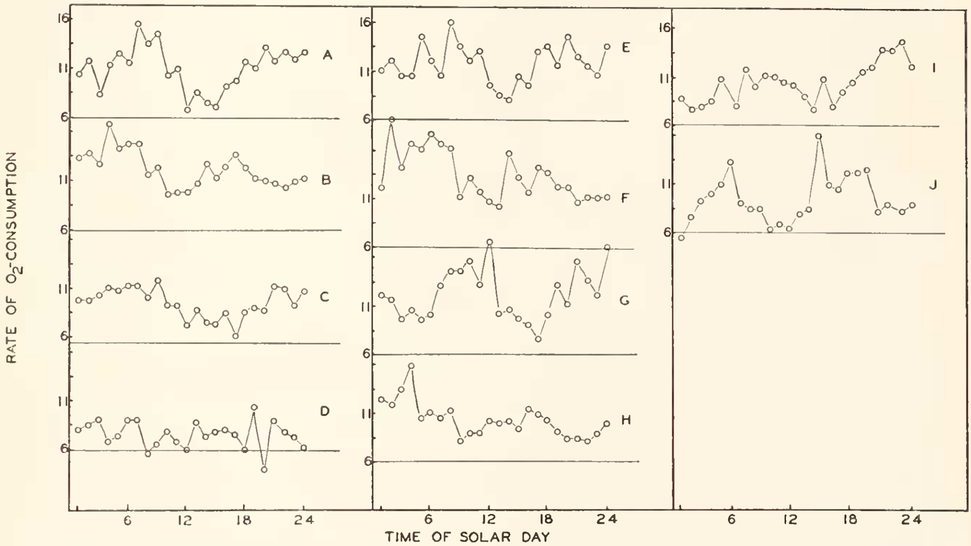

FIGURE 3. Variation in O_2-consumption of *Uca pugnax* on single days during the summer of 1957. See text for further explanation.

maxima. The respiratory pattern is thus repeated at approximately 15-day intervals.

Although the major maxima and minima are, in general, readily distinguished in Figure 3, it is seen that on many days single points appear which deviate widely from the trend of the series of points around them. Such points may make difficult the comparison of the form of curves, especially on days on which the amplitude of fluctuation is low. Another aspect of the data that might contribute to the difficulty of analysis of form is the day-to-day variation in the level at which O_2-consumption occurs. Such variation might permit a single day with high values to contribute disproportionately to the form of a mean curve for periods of several days taken together. The first difficulty can be minimized by the use of successive, overlapping three-hour averages to obtain the hourly values. The effect of variation in level of O_2-consumption can be reduced by using the ratio of hourly values to mean value for the day.

Evidence that the use of three-hour overlapping averages preserves the form and phase relations of fluctuations occurring over a period of four or more hours, while eliminating the irregularities of single hours, is presented in Figure 4. In this figure are plotted (A) the primary lunar curve for the period July 14 to August 12 obtained by converting the raw hourly data to the ratio of hourly value to mean for the day and then rendering random the diurnal variations in the man-

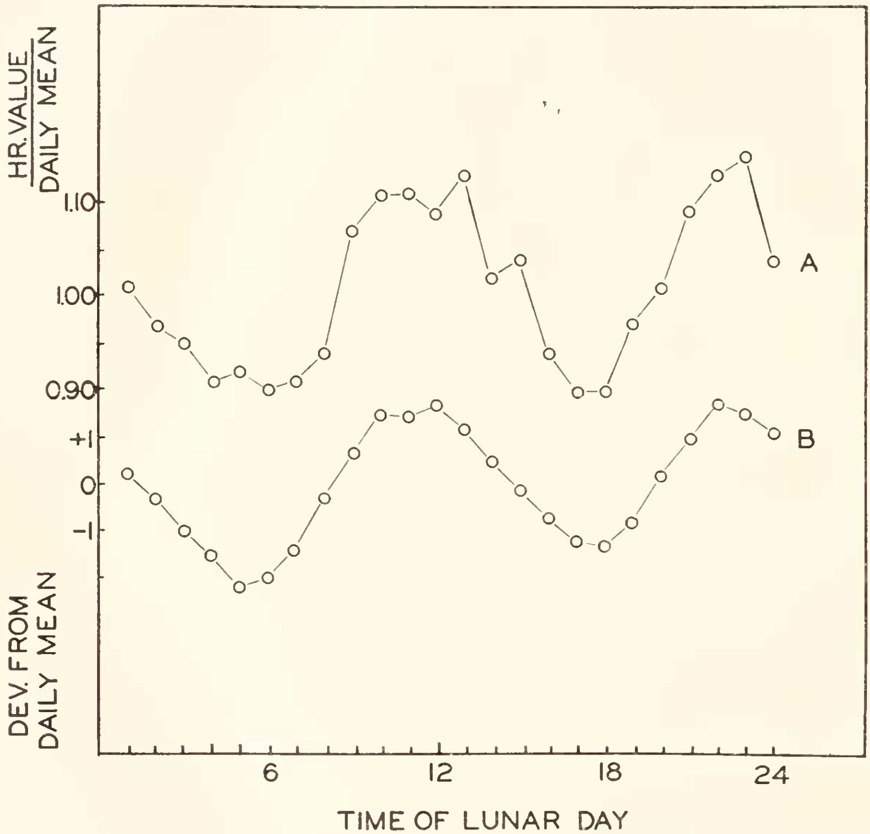

FIGURE 4. The primary lunar rhythm in O_2-consumption of *Uca pugnax*. Curve A presents raw data, Curve B presents data smoothed by use of overlapping 3-hour averages.

ner previously described. Curve B, Figure 4, shows the primary lunar curve obtained by use of overlapping three-hour averages for hourly values. The data are for the same period as those in Curve A. (Figure 4B is the same curve as was seen in Figure 2C.)

As might reasonably have been expected, Curve B is smoother than Curve A—that is, there is some loss of sharpness of definition so far as points of transition are concerned. It is quite clear, however, that if one is concerned with the form

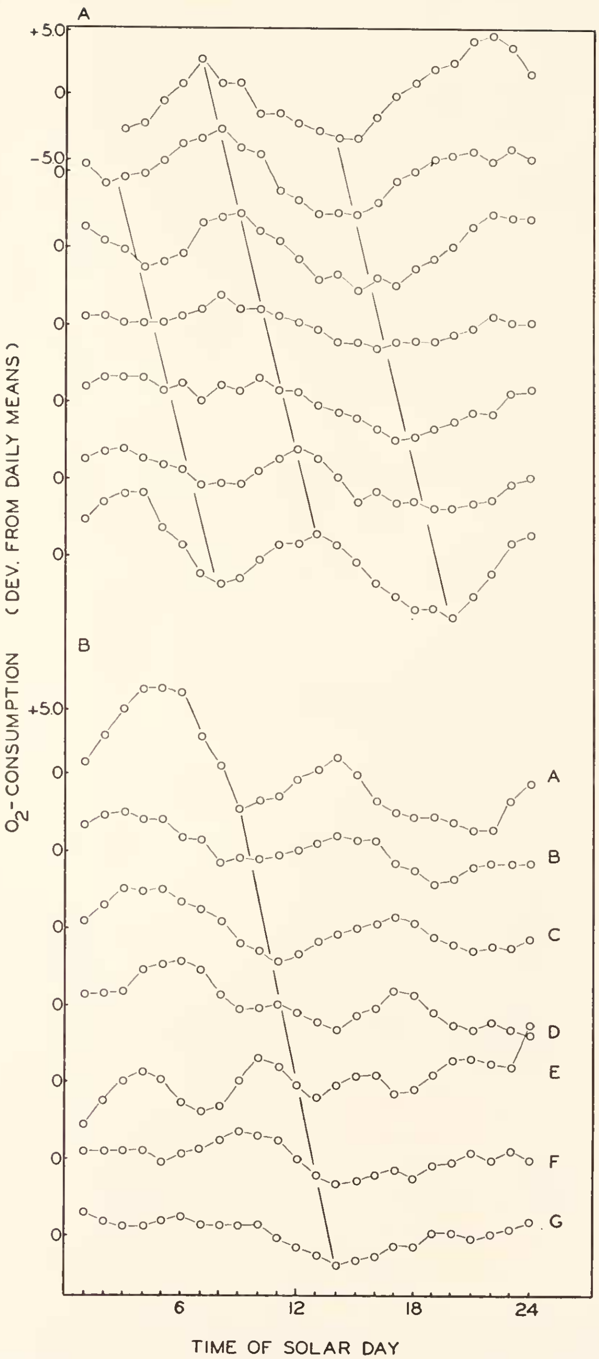

FIGURE 5. Variations in O_2-consumption of *Uca pugnax* for two successive 7-day periods, (A) June 23 to June 29, and (B) June 30 to July 6, 1957. The ordinate scale used throughout the figure is that indicated for the top curves.

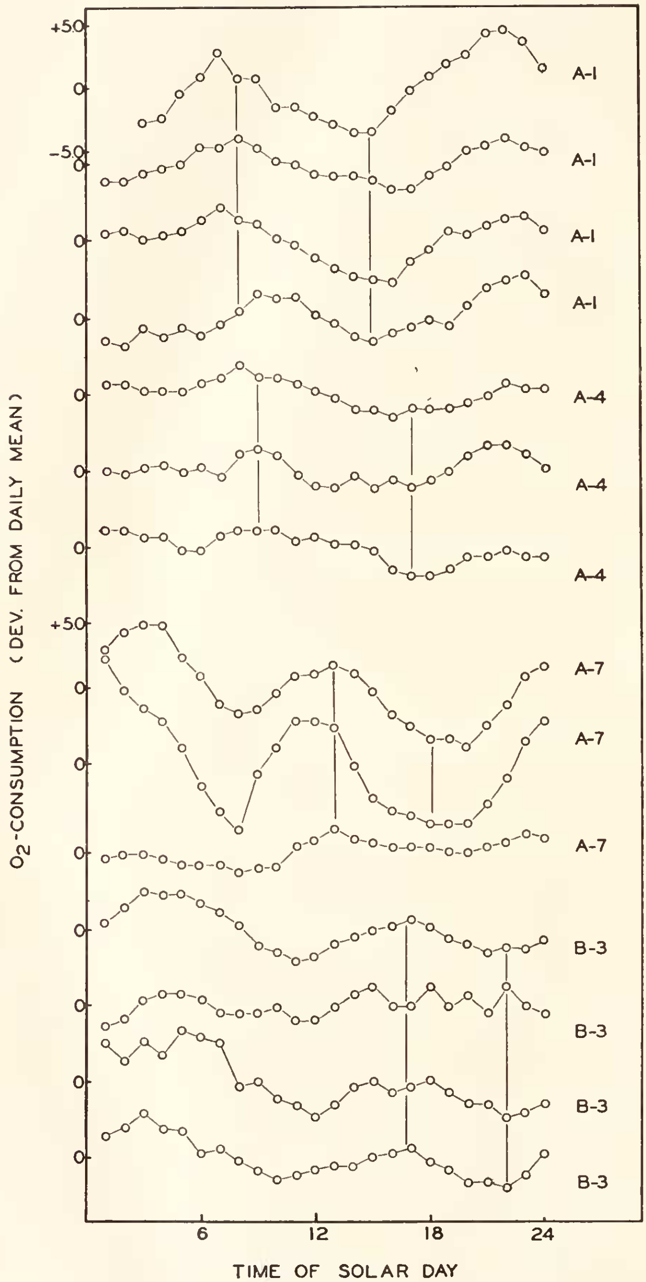

FIGURE 6. Variations in pattern of O_2-consumption throughout semi-lunar period as shown by the recurrence of form on comparable days. The ordinate scale used throughout the figure is that indicated for the top curve.

and placement of major fluctuations occurring over periods of several hours Curve B is adequate. If one is concerned with the precise difference between adjacent hours Curve A would be preferable. Since we are in the present work interested only in fluctuations with periods of 12 to 24 hours we shall use overlapping three-hour means in the analyses of the data.

The day-to-day changes in pattern associated with the overt lunar rhythm are illustrated in Figure 5 which shows hourly data for successive single days. Each point represents the mean hourly value for all recorders operating on that day, the values being expressed as deviations from the mean for that day.

In Figure 5A are seen the O_2-consumption data for the seven days beginning June 23, 1957 (top curve) and ending June 29, 1957 (lowest curve). The diagonal lines indicate progression of maxima and minima across the day. It can be seen that both maximum and minimum have advanced 6 hours in the 7 days illustrated. This is a rate of 51.4 minutes per day. This is to be compared with about 50 minutes per day, the rate of lunar progression. In the period represented, new moon occurred on the fifth day.

In Figure 5B are seen data for the seven days immediately following those in 5A, *i.e.*, June 30 to July 6 inclusive. It is seen that only the first four of these curves are clearly bimodal. Moreover in these first four there does not appear the clear progression of a peak through the day that was observed in the preceding seven days. In Curve A, Figure 5B, two maxima are obvious, one at about 5 AM and the second at 2 PM. Both maxima seem to have disappeared in the last three curves of the series. The minimum present in Curve A can be identified with one present in each of the last three, as is indicated by the diagonal line. The rate of progression of this minimum is such that in 7 days it has moved 5 hours, or about 43 minutes per day. In the two 7-day periods represented in Figure 5, a single minimum has progressed 11 hours in 14 days, at the over-all rate of 47.2 minutes per day, which is quite good agreement with the average rate of lunar progression.

To demonstrate the recurrence of characteristic forms of the daily pattern of respiration at comparable times in a semi-lunar period, Figure 6 has been prepared. For convenience of description and labelling a lunar period is divided into units of 7 or 8 days each as follows: June 23 to June 29 is called an A period, consists of 7 days of which the fifth is the day of new moon. The period from June 30 through July 6 is a B period and is 7 days long. From July 7 to 13 inclusive is again an A period, is 7 days long, and full moon occurs on the fifth day. Continuing through the summer in the same manner, A periods are always 7 days long and new or full moon occurs on the fifth day. The intervening days are included in the B periods which may be either 7 or 8 days, depending on the number of days available. In this way any given day of a semi-lunar period can be identified by a letter and a number. The days so represented in Figure 6 are: A-1, A-4, A-7, and B-3. Each group of days synchronous with respect to semi-lunar period is indicated by two vertical lines connecting members of a group at maxima and minima.

The first group of curves in Figure 6, representing four A-1 days during the summer of 1957, shows quite clearly the resemblance among A-1 days even though separated in time by as much as two months. All of the other groups also reveal great internal similarity. Moreover, any member of A-1 is more nearly like any member of A-4 than like any member of A-7 or B-3, regardless of the absolute

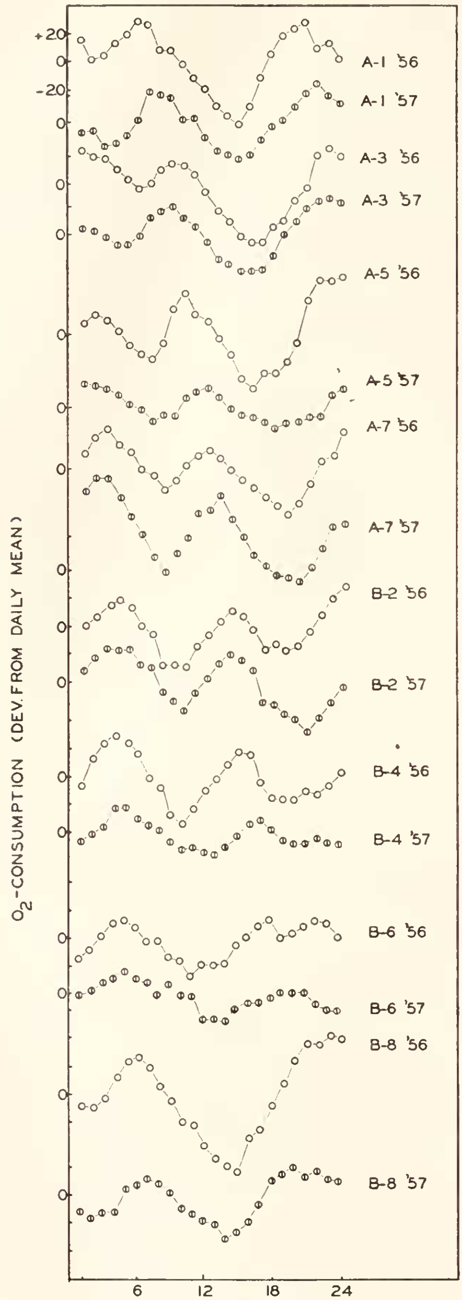

FIGURE 7. Comparison of changes in pattern of respiration throughout mean semi-lunar period as obtained in 1956 (upper member of each pair) with those obtained in 1957 (lower member of each pair). The ordinate scale used throughout the figure is that indicated for the top curve.

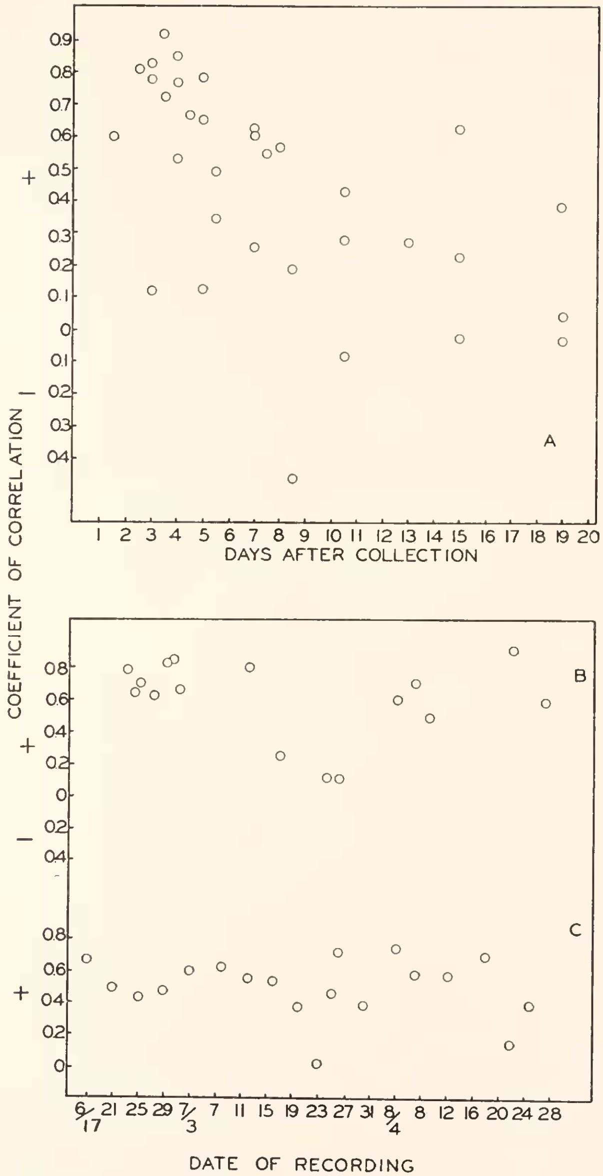

FIGURE 8. Coefficients of correlation of simultaneously recorded values of O_2-consumption for different groups of *Uca pugnax*, (A) as a function of duration of stay in laboratory, (B) as a function of date of collection, (A and B for 1957), and (C) as a function of date of collection (data from 1956).

time difference between the curves compared. The progression of maxima and minima throughout the day seen in Figure 5 for a single semi-lunar period is seen, in Figure 6, to have been repeated throughout the entire summer.

The striking similarity of the form of curves representing comparable times of the semi-lunar period is further illustrated by a comparison of the results obtained in different years. In Figure 7 are plotted the mean curves for every second day of a semi-lunar period. The points were calculated as deviations from daily means. The top curve of each pair represents the mean of all the indicated days from 1956, the lower member of each pair the mean of comparable days from 1957. With respect to form of the curve and the placement of major maxima and minima the curves for the two years are practically indistinguishable. It is thus clear that a characteristic pattern of fluctuations in respiratory rate is exhibited by *Uca pugnax* and that, although the pattern varies in a regular manner with the semi-lunar period, both the basic pattern and its regular variations were almost identical in the summers of 1956 and 1957.

With the establishment of the existence of a regularly repeating pattern of respiration it became of interest to investigate the extent of agreement within groups of animals at any one time. For this purpose correlations of simultaneous hourly values for O_2-consumption of different groups of animals were performed. The coefficients of correlation were calculated for limited periods of one to three days and were calculated separately for data from animals collected at different times. This method of analysis permitted comparison of results in terms of the actual dates of recording, the duration of time in the laboratory, and the dates of collection. The resulting comparisons are presented in Figure 8 and in Table I.

Table I

Comparison of simultaneously recorded respiratory values

Collection date	Coefficients of correlation Days after collection						
	1	3	4	5	7	8	15–17
6/20		+0.779	+0.652	+0.700	+0.625	+0.565	
6/26		+0.825	+0.849	+0.666			+0.226 ±0.12
7/10		+0.808			+0.256 ±0.11	−0.464 ±0.11	−0.022 ±0.12
7/21		+0.120 ±0.10		+0.125 ±0.10		+0.185 ±0.10	
8/3	+0.600		+0.726	+0.490 ±0.06		+0.545	+0.618
8/20		+0.915			+0.602		

In Figure 8A the coefficients of correlation for all of the data obtained in the summer of 1957 are plotted as ordinate values with the number of days in the laboratory along the abscissa. There is, in general, a decrease in correlation with time in the laboratory, such that after eight days the values approach a random distribution. It is also obvious that there is a high degree of scattering of values even when the number of days in the laboratory is small.

Figure 8B shows the coefficients of correlation for simultaneous hourly values of O_2-consumption obtained within the first seven days after collection. These values are plotted as a function of the date on which respiration was recorded. In this graph the low values are clearly grouped in the last two weeks of July while strong positive correlations are found during the rest of the summer.

In Figure 8C are plotted similar coefficients of correlation calculated from the data for the summer of 1956. During that summer animals were collected weekly and none were used after being in the laboratory for seven days. Here, too, there is a drop in the value of the coefficients of correlation during the same period for which it was found in 1957. During 1956 there was, in addition, a reduction in correlation in the latter part of August. Unfortunately there are insufficient data for these dates in 1957 to confirm or deny the existence of a similar reduction in that year.

Table II

Correlation of hourly values for successive semi-lunar periods

Day of semi-lunar period	Coefficients of correlation 1956	1957
A-1	+0.232±0.10	+0.550±0.08
A-2	+0.618±0.06	+0.485±0.08
A-3	+0.568±0.07	+0.667±0.05
A-4	*+0.075±0.14*	+0.530±0.07
A-5	+0.455±0.09	*+0.040±0.10*
A-6	+0.610±0.06	+0.402±0.08
A-7	*+0.150±0.12*	+0.428±0.09
B-1	+0.518±0.08	+0.430±0.09
B-2	+0.390±0.12	+0.502±0.09
B-3	+0.660±0.06	+0.360±0.09
B-4	+0.230±0.11	+0.610±0.06
B-5	—	+0.440±0.08
B-6	*+0.100±0.11*	*+0.020±0.10*
B-7	+0.440±0.09	+0.430±0.08
B-8	+0.440±0.12	+0.740±0.06

In Table I are recorded values for the coefficients of correlation from each collection made during 1957. The coefficients are listed as obtained for various short intervals after collection up to eight days and for the fifteenth and seventeenth days after collection. A comparison of the values for the third day after collection shows that the only group of animals not showing a strong positive correlation, highly significantly different from zero, was the one collected on July 21. It is also of considerable interest that the group collected on July 10, although showing the usual strong positive correlation on the third day, no longer shows a significant degree of correlation by the seventh day and actually shows a significant negative correlation on the eighth day. This is in marked contrast with the collections of June 20 and August 3. In this latter case a strong positive correlation was still found at seventeen days after collection. It appears, then, that considerable variation in both the initial coefficient of correlation and the rate of decrease of the coefficient is found among the various groups of animals. The variations observed are not obviously related either to phase of moon or to treatment related to maintenance in the laboratory. The only factor that seems to give any system to these variations is the date of recording respiration.

Coefficients of correlation were also calculated for the hourly values on one day of a semi-lunar period with the hourly values for each of the comparable days of successive semi-lunar periods throughout the summer. These provide a measure of the similarity between comparable days in successive semi-lunar periods for five such periods for the summer of 1957. These values are presented in Table II from which it is seen that for thirteen of the fifteen days, coefficients ranging from 0.358 to 0.740 were obtained. For the other two days the coefficients of correlation were found to be not significantly different from zero. The two days for which significant positive correlations did not obtain were the day of new or full moon (day A-5) and the eighth day after new or full moon (day B-6). Similar coefficients of correlation were calculated from the data for 1956 and are included in Table II. It will be observed that of the days for which sufficient data were available to permit making the correlations, days B-6, A-4, and A-7 yielded coefficients not significantly different from zero.

Discussion

Two apparently conflicting characteristics of the behavior of *Uca pugnax* emerge from the results reported in this paper. We find that when population samples, ranging in size from 8 to 24 animals, are compared at 15-, 30-, and 45-day intervals the pattern of respiration is being reproduced almost identically every 15 days. When data from somewhat larger numbers of animals are compared from year to year the same precise reproduction of pattern is observed. However, when a group of four animals is compared with two to five other groups of four animals at the same time, as was done by the correlation of simultaneous hourly values, extreme variability is found. It should be emphasized that there are at least three possible situations, all of which would result in a lack of good correlation. One possibility, of course, is that there are in fact only negligible or random fluctuations in the respiratory rate of all animals. This possibility can almost certainly be excluded. In the latter part of July when conspicuously low coefficients of correlation were obtained, the mean daily curves were of normal amplitude and phase relations. An example of this can be seen in Figure 3, curve E, the points of which represent the mean hourly values for July 23.

A second situation that would lead to poor correlation among simultaneous hourly values would occur if one or two machines were recording the normal rhythmic pattern while three or four were producing non-rhythmic fluctuations or no significant fluctuations.

The third possibility leading to lack of correlation is that different samples, while still rhythmic, have become out of phase. Examination of the individual records revealed one two-day period (July 18–19) when two recorders showed typical high-amplitude fluctuations but with one recorder almost precisely in opposite phase to that of the other. This situation is reflected in the large negative correlation (−0.464) recorded in Table I for the collection of July 10. In no other case was a situation of this type obvious. However, it is recognized that if the individuals making up a sample of four were out of phase with each other the record would be indistinguishable from that produced by four non-rhythmic individuals. The multiple peaks evident in Figure 5B, Curves D and E, may indicate such a lack of synchrony among individuals within samples.

Even though there is no way of distinguishing between a loss of rhythm by part of the population and a loss of synchrony, the fact remains that a sufficiently great proportion of the population retains a rhythm in normal phase relations to impart to the mean daily curves a striking regularity. The question of the basis for this regularity represents one of the fundamental problems in biological rhythms.

For the study of this basic problem there are certain distinct advantages to be gained by examining rhythms with periods other than twenty-four hours. If the rhythm being studied is clearly of semi-lunar (or tidal) frequency there can be no question of induction by fluctuations in environmental factors associated with solar day-night. There is similarly little probability that the normal activity in the laboratory where the animals are kept will exhibit tidal-frequency fluctuations. We are faced then with the situation that a predominant part of the population can maintain the rhythm of O_2-consumption with almost absolute precision for as long as 16 days away from the ordinary tidal influences. This occurred while the animals simultaneously exhibited a diurnal rhythm of O_2-consumption. Either one must attribute a remarkable precision to a biological system in a situation not constant but quite different from the normal habitat of the animals, or one must invoke environmental factors not universally accepted as constituting stimuli for the organisms concerned. The first alternative implies, on the part of the organism, a degree of detachment from the environment that is not entirely acceptable to modern biological thought. The second alternative permits an acceptable degree of dependence upon the environment but requires the recognition of hitherto unsuspected stimulating factors.

Summary

1. The form of the mean diurnal rhythm of O_2-consumption of *Uca pugnax* is described and found to be practically identical for the summers of 1955, 1956, and 1957.

2. The mean lunar-day rhythm of O_2-consumption is described for the summers of the same three years. The curve for any one of these years is indistinguishable from that for either of the other two years.

3. The mean lunar-day rhythm consists of two maxima, of equal magnitude, occurring approximately at lunar zenith and at lunar nadir; between the maxima are two minima symmetrical with respect to time of occurrence and magnitude.

4. For the primary lunar rhythm the ratio of maximum to minimum is about 1.4; for the diurnal rhythm the ratio of maximum to minimum is 1.2 in 1955, 1.4 in 1956, and 1.2 in 1957.

5. Because of the amplitude of the lunar component of the rhythm, the data for single days reveal clearly the progression of lunar maxima and minima.

6. Because of the equality in amplitude of fluctuations correlated in time with lunar zenith and with lunar nadir, the overt rhythm is one with a period of 12.4 hours. There is a pattern of fluctuations characteristic of each day in a semi-lunar period.

7. The reproducibility of the daily pattern in successive semi-lunar periods, and in successive years, is demonstrated.

8. In general a strongly positive correlation is found between simultaneous hourly values for different groups of animals during the first seven days after col-

lection. There is a general decrease in the extent of these correlations with time in the laboratory.

9. There is strong evidence for a time-dependent variable affecting the size of the coefficients of correlation for simultaneous hourly values obtained during a wide range of times in the laboratory.

10. Hourly values for single days of a semi-lunar period were correlated with the hourly values for comparable days of successive semi-lunar periods. The coefficients of correlation were positive and significantly different from zero for thirteen of the fifteen days of 1957, the exceptions being the eighth day after new or full moon and the day of new or full moon for which the coefficients were not significantly different from zero. For 1956 the exceptions were the day before new or full moon, and the second and eighth days after; the coefficients for these three days were not significantly different from zero. For the other twelve days the correlations were strongly positive.

11. The relevance of these findings to an understanding of the phenomenon of biological rhythmicity is discussed.

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